Decomposition of loblolly pine bark by soil- and root-inhabiting fungi

E. G. KUHLMAN
Southeastern Forest Experiment Station, United States Department of Agriculture,
Research Triangle Park, North Carolina

Received March 23, 1970

KUHLMAN, E. G. 1970. Decomposition of loblolly pine bark by soil- and root-inhabiting fungi. Can. J. Bot. **48**: 1787–1793.

A modified soil-block test was used to compare the bark-decomposing ability of various soil- and root-inhabiting fungi. Bark of *Pinus taeda* was highly resistant to decomposition by all 31 fungi tested. A brown-rot fungus, *Lenzites saepiaria*, caused the most weight loss, but weight losses due to decay by all fungi varied only from 3 to 15%. Isolates of Mucorales produced 3–8% weight losses from stem bark in 12 weeks. Available nutrients were used within 6 weeks; longer incubation resulted in little additional decomposition. Losses in weight from root bark and stem bark were similar, indicating little nutritional difference between these two substrates.

Extraction of stem bark with ethanol or water before incubation with *Fomes annosus*, *L. saepiaria*, or *Scytalidium lignicola* did not increase the amount of decomposition. This suggests that extractives in the bark may not be responsible for the slow rate of decay. Since autoclaving of the bark before incubation with the fungi enabled the fungi to cause more weight loss than did gas sterilization of the bark, the primary reason for the slow rate of decomposition by fungi is considered to be the complex molecular structure of the bark constituents.

Introduction

Mycelium of *Fomes annosus* (Fr.) Karst. occurs among the bark scales of pine roots up to 1 m in advance of its growth through the wood (8, 11, 23). This epiphytic growth habit enables the fungus to establish new infections on the same root or at points of contact with roots of other trees. *F. annosus*, along with many other fungi, can be isolated from root bark in all stages of root decay. In spite of an abundant fungal population the bark appears very resistant to decay and remains intact after the wood is decomposed. However, little is known about the capacity of *F. annosus* and other root- and soil-inhabiting fungi to use root bark as a substrate for growth and survival.

This study was undertaken to compare the in vitro bark-decomposing ability of various soil- and root-inhabiting fungi to determine if the bark is a major substrate for growth and survival of *F. annosus*. Three hypotheses concerning the resistant nature of the bark were tested.

Methods

The fungi used in these experiments were isolated from forest soil or root bark and wood of loblolly (*Pinus taeda* L.) and slash pine (*P. elliottii* Englm. var. *elliottii*) (15) (Table III). Three wood-destroying fungi (two of the white-rot type, *F. annosus* and *Peniophora gigantea*, and one of the brown-rot type, *Lenzites saepiaria*) were used for comparison with species of Mucorales and Fungi Imperfecti.

The relative bark-decomposing ability of the fungi was determined on the basis of weight loss using the ASTM standard soil-block test (2) with these modifications: bark discs were used rather than wooden test blocks; in three experiments the bark was placed in the bottles before, rather than after, autoclaving of the decay chambers; and the feeder blocks were not used in some experiments. All decay chambers were dipped in a solution containing 260 p.p.m. Kelthane after inoculation to prevent contamination by mites.

The bark for each study was taken from a 25- to 30-year-old loblolly pine growing at Research Triangle Park, N.C. Stem bark was from the lower 6 ft of the trunk, and root bark was from main laterals within 2 ft of the root collar. Discs were removed with a 12-mm plug cutter oriented perpendicularly to the bark surface and penetrating down to but excluding the cambium. Discs of root bark were 4–8 mm long; those of stem bark were all 12 mm long. The following separate studies were conducted.

1. Decomposition of stem and root bark by *F. annosus* and *P. gigantea* both with and without wooden feeder blocks present as an additional source of nutrients. The eight treatments were replicated six times and incubated for 12 weeks.
2. Decomposition of stem bark by 19 isolates of mucoraceous fungi during a 12-week incubation without feeder blocks. Each isolate was replicated six times although contamination reduced this number in some cases.
3. Decomposition of stem bark by 15 fungi from several taxonomic groups for incubation periods of 6, 12, and 18 weeks. Each species and incubation time was replicated four times and wooden feeder blocks were used.
4. The effect of treatment before incubation on decomposition of stem bark by *F. annosus*, *L. saepiaria*, and *Scytalidium lignicola* during a 12-week incubation period without feeder blocks. The treatments were as follows: (1) exhaustive extraction with 95% ethanol and sterilization by autoclaving, (2) exhaustive extraction with

distilled water and sterilization by autoclaving, (3) nonextracted and autoclaved, and (4) nonextracted and sterilized with ethylene oxide. The bark was sterilized separately from the soil which was autoclaved for all treatments. Each treatment was replicated six times for each fungus.

Decomposition was calculated as the average percentage weight lost by the replicate discs on an oven-dry basis. Analysis of variance was used to determine significance among treatment means. Multiple comparisons among treatment means were made with Tukey's W-procedure for honestly significant differences (h.s.d.) (26) in experiments 1, 2, and 4. Scheffe's S-method (24) of determining error rate was applied in experiment 3.

Exhaustive solvent extractions with ethanol or water were carried out for 168 h in a Soxhlet apparatus on bark discs or bark ground to pass a 40-mesh screen. Nonexhaustive extractions were for 24 h on ground bark. The TAPPI Standard T4m (27) was used to determine solubility in 1% sodium hydroxide, except the samples initially weighed 100–600 mg instead of 2 g. Readily available carbohydrates were analyzed by the method of Pasternack and Danbury (20) in study 4 from noninoculated discs and from discs decayed by the fungi. Total nitrogen and carbon were determined in a Coleman Nitrogen Analyzer and a Fisher Induction Carbon Apparatus.

To determine if autoclaving released extractable compounds, fresh bark was ground and subjected to exhaustive extraction with ethanol or water. Portions of each residue were air-dried and oven-dried. The oven-dried portion was further divided and subportions were treated as follows: not autoclaved, autoclaved for 45 min, autoclaved for two 45-min periods, and gas-sterilized. All portions were then subjected to nonexhaustive extraction, first with the original solvent and then with the other solvent.

Results and Discussion

Root bark and stem bark of loblolly pine were equally resistant to decomposition by either *F. annosus* or *P. gigantea* (Table I). Stem bark was used in later experiments because it provided a larger and more uniform sample. The presence or absence of wooden feeder blocks below the bark discs as accessory sources of nutrients had little effect on decomposition. The similarity in decomposition of stem and root bark contrasts with the uniformly greater decomposition of root wood than of stem wood of loblolly pine and seven other coniferous species (22).

TABLE I

Weight loss due to decay of root and stem bark of loblolly pine by *Fomes annosus* and *Peniophora gigantea* in 12 weeks with and without feeder blocks

		Average weight loss, %[a]	
Fungus	Feeder block	Root bark	Stem bark
Fomes annosus	Present	6.5	8.0
Fomes annosus	Absent	7.2	7.3
Peniophora gigantea	Present	6.0	7.6
Peniophora gigantea	Absent	8.7	7.7

[a] Average of six replicates; honestly significant difference (h.s.d.)$_{.05}$ > 2.36.

The wood-rotting fungi caused only a 6–15% weight loss from loblolly pine bark (Tables I, III, IV). This rate of decay is low, although for *F. annosus* it is similar to the 14% weight loss reported from stem sapwood of loblolly pine in 12 weeks (22). Allison and Murphy (1) reported that with a natural soil mycoflora, decomposition of finely ground bark of southern pines was about one-half the decomposition of sawdust from the same trees when measured by CO_2 evolution during a 60-day period. In contrast, during a 30-day period bark and sawdust decomposition of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, was about equal (3).

Since the wood-destroying fungi did not efficiently decompose bark, it is interesting that the species of Mucorales and Fungi Imperfecti tested were able to cause 3–9% weight loss (Tables II and III). Previously, Duncan (7)

TABLE II

Weight loss due to decay of stem bark of loblolly pine by various Mucorales in 12 weeks

Isolate	Reps., No.	Av. wt. loss, %[a]
Zygorhynchus moelleri Vuil.	3	8.2
Mortierella ramanniana (Moll.) Linnemann	6	7.8
Gongronella butleri (Lendn.) Peyronel & Dal Vesco	3	7.0
Mortierella pulchella Linnemann	2	6.9
Absidia coerulea Bainier	4	6.7
Mortierella marburgensis Linnemann	6	6.7
Mucor lamprosorus Lendner	6	6.5
Mortierella bainieri Costantin	6	6.3
Mucor spinescens Lendner	6	6.0
Mortierella elongata Linnemann	6	5.9
Mucor hiemalis Wehmer	4	5.7
Mortierella minutimissima Van Tiegham	5	5.6
M. marburgensis	5	5.4
Mortierella candelabrum Van T. & LeMon.	6	5.4
Mortierella nana Linnemann	5	4.9
Mortierella jenkini (Smith) Naumov	5	4.9
Mortierella zychae Linnemann	6	4.6
M. candelabrum	6	3.9
Mortierella verticillata Linnemann	6	3.1

[a] h.s.d.$_{.05}$ > 3.74; weighted for three vs. six replicates > 4.58.

showed that most Fungi Imperfecti isolated from wood in use were unable to decompose southern pine sapwood during a 9-month period, although some caused weight losses of up to 24%.

Moisture stress should not have been a factor in the low weight losses reported. All fungi grew over the surface of the bark. Some isolates produced abundant vegetative mycelium, while others (e.g., *Gliocladium deliquescens*) sporulated without abundant mycelium. In the fourth experiment the moisture content of the bark after incubation was found to vary from 66 to 144%. Thus, the moisture content was considerably above the equilibrium moisture content (Table VI) and within the range of moisture contents suggested as acceptable for wood decomposition (21).

A small fraction of loblolly pine bark is susceptible to rapid degradation by all fungi tested, as indicated by the uniform weight loss caused by all 15 species after 6 weeks (Table III). The bulk of the bark constituents were highly resistant to decay and there was no increased weight loss with incubation periods of 12 or 18 weeks as would occur with wood.

Three hypotheses were formulated to explain the uniformly low weight losses. (1) Carbohydrates in the bark were degraded readily by all fungi, but other substrates in the bark were resistant. (2) Toxic or inhibitory materials present in bark reduced or limited growth of all fungi. Toxic extractible substances are the principal source of decay resistance in heartwood of certain species. Extraction of these substances with hot water or organic solvents can reduce the decay resistance of heartwood (25). (3) The complex molecular structure of the bark limited its breakdown by fungi. Lignin, for example, contains many linkages that require specific enzymes for cleavage. Thus, lignification limits breakdown of wood to organisms with those specific enzymes (25). Bark may also contain high-molecular weight, insoluble polymers whose structural complexity make degradation a slow process.

"Readily available" carbohydrates constituted 3–5% of the oven-dry weight of the bark in all samples tested. This is similar to the 4% carbohydrate reserve reported for outer bark of shortleaf pine, *P. echinata* Mill. (10). The percentage of carbohydrates in the bark discs

TABLE III

Weight loss due to decay of stem bark of loblolly pine by various fungi in 6, 12, and 18 weeks

		% loss[a]			
		Weeks of incubation			% loss,[b] mean for isolate
Isolate	Source of isolate	6	12	18	
Lenzites saepiaria (Wulf.) Fr.	Pine wood	12.3	9.4	13.1	11.6
Peniophora gigantea (Fr.) Massee	Pine wood	8.8	7.7	9.6	8.7
Trichoderma polysporum (Link ex Pers.) Rifai	Forest soil	7.1	9.4	8.9	8.5
Geotrichum sp.	Pine wood	7.0	6.4	8.6	7.4
Fomes annosus	Pine wood	9.5	6.4	6.1	7.3
Sterile FSL 256	Pine bark	3.2	8.1	9.2	6.8
Sterile FSL 257	Pine wood	6.2	6.2	7.7	6.7
Penicillium restrictum Gilman & Abbott	Forest soil	5.5	5.2	9.0	6.6
Chloridium chlamydosporis (van Beyma) Hughes	Pine bark	5.1	7.0	5.9	6.0
Scytalidium lignicola Pesante	Pine wood	6.0	6.0	5.7	5.9
Trichoderma harzianum Rifai	Forest soil	4.3	5.6	7.6	5.8
Verticicladiella sp.	Pine bark	4.4	6.8	5.8	5.7
Gongronella butleri	Pine bark	4.1	6.0	6.5	5.5
Gliocladium roseum (Link) Bainier	Forest soil	4.5	3.9	5.8	4.8
Gliocladium deliquescens Sopp	Pine bark	3.3	4.0	3.2	3.5
Mean for time[c]		6.1	6.5	7.5	

[a] Treatment > 11.5, $n = 4$.
[b] Isolate > 4.20.
[c] Time > 1.05.
NOTE: 5% error rate for differences between means.

was not changed significantly by extraction with hot water or ethanol or by incubation with the fungi. The carbohydrates may be located within heavily suberized cells that were impervious to the solvents and the fungi, and were released only upon grinding in the Wiley mill and freezing during the carbohydrate analysis. Therefore, the "readily available" carbohydrates were not the substrates used by the fungi.

Exhaustive extraction of the bark discs with water or ethanol before incubation with *F. annosus*, *L. saepiaria*, or *S. lignicola* did not markedly change the rate of decomposition of bark by these three fungi (Table IV). However, *Lenzites saepiaria* produced significantly less weight loss from bark previously extracted with water than it did from nonextracted bark, and *Fomes annosus* caused less weight loss from water-extracted bark than from ethanol-extracted bark. In the case of these three fungi, the ethanol and water extractives did not inhibit decomposition of bark; a portion of the water extractives was used as a substrate for growth of *L. saepiaria* and *F. annosus*. Unless extractives soluble in other solvents are inhibitory to growth of fungi, it does not appear that toxic extractives prevent decomposition of loblolly pine bark by fungi.

The complex molecular structure of the bark appears to limit breakdown by fungi. Discs of bark that were not extracted and were sterilized with gas had the least decomposition by all three fungi (Table IV). This suggested that autoclaving the bark made some substrates available to the fungi that were not available in the gas-sterilized treatment. This possibility also was indicated by re-extracting the water- and ethanol-extracted bark after the incubation period and after grinding in the Wiley mill (Fig. 1). Both noninoculated check treatments had slightly more extractives present after autoclaving than they had in the first extraction. Similar results were obtained with fresh bark ground before exhaustive extraction and then re-extracted after being oven-dried and autoclaved for 45 min (Fig. 2). Autoclaving of the ground bark for 45 min released the same quantity of extractives as had been removed during the first extraction.

The air-dried bark contained additional extractives, although not as much as the samples that were autoclaved (Fig. 2). Some workers (12, 28) immerse bark in methanol or boiling ethanol after separation from the tree to prevent enzymatic, chemical, or biological changes. These changes must have occurred in the air-dried sample. Nevertheless, the amounts of water and ethanol extractives reported in this study for loblolly pine bark are similar to those in previous reports for other pine species (5, 19).

The total water and ethanol extractives present in bark after incubation with *F. annosus*, a white-rot fungus, were greater than the total extractives present after incubation with *L. saepiaria*, a brown-rot fungus (Fig. 1). This contrast was especially marked in samples extracted with ethanol before incubation with the fungi. White-rot fungi apparently use partial degradation products from wood more efficiently than those from bark. Campbell (4) and Cowling (6) have demonstrated that the amount of soluble material in white-rotted wood is essentially constant, whereas brown-rotted wood generally has increasing quantities of soluble extractives with increasing weight loss. Cowling (6) suggested this was due to similar rates of depolymerization and respiration of depolymerization products by white-rot fungi, whereas

TABLE IV

Weight loss due to decay of stem bark of loblolly pine by various fungi in 12 weeks as affected by previous extraction or method of sterilization

	Autoclave-sterilized			Gas-sterilized
Fungus	Water-extracted	Ethanol-extracted	Non-extracted	Non-extracted
Fomes annosus	12.7	14.8	14.2	7.3
Lenzites saepiaria	10.7	13.7	15.3	8.8
Scytalidium lignicola	11.2	12.5	12.5	8.6

NOTE: h.s.d.$_{.05}$ treatment means = 2.1.

brown-rot fungi depolymerized the wood constituents faster than they respired the breakdown products. In bark the opposite may be true. Hata *et al.* (9) have shown that the white-rot fungi, *F. annosus* and *F. fomentarius* (Fr.) Kickx., grew more slowly and caused less decomposition of bark phenolic acids than of bark or wood MW lignins. Their results also indicated that bark MW lignins were used somewhat less readily than wood MW lignins. In preparing the MW lignins, Hata *et al.* (9) depolymerized polymers that white-rot fungi may not be able to cleave *in situ.* In terms of chemical properties, bark lignin and wood lignin differ considerably (13). This is evident by differences in solubility and by the considerably more heterogeneous composition of bark lignin than of wood lignin (13). Therefore, even though 50–80% of the outer bark is lignin (12, 28), bark lignin may be different enough from wood lignin to resist degradation by white-rot fungi. The brown-rot fungus, *L. saepiaria*, apparently used some constituents in bark very efficiently without leaving many breakdown products.

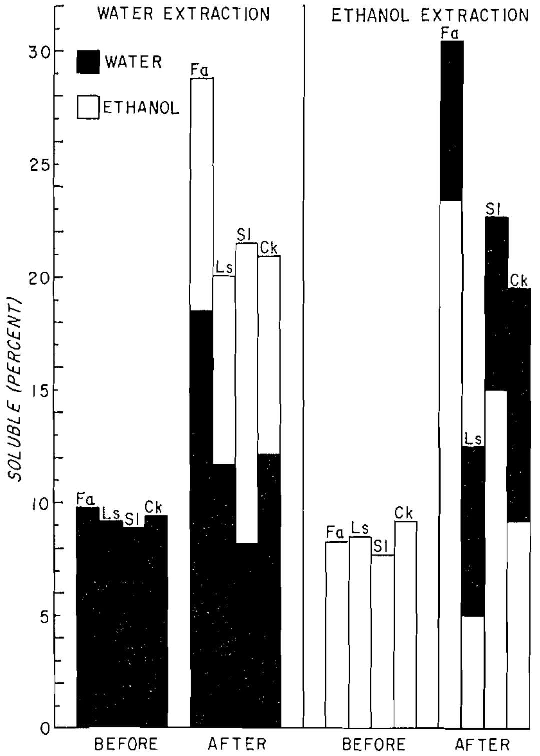

FIG. 1. Percentage water or ethanol extractives present in loblolly pine bark before and after incubation for 12 weeks with *Fomes annosus* (Fa), *Lenzites saepiaria* (Ls), *Scytalidium lignicola* (Sl), or non-check (Ck).

"Bark lignin" or "bark phenolic acids" are soluble in 1% sodium hydroxide. Wood decayed by brown-rot fungi generally has more alkali-soluble materials than white-rotted wood. However, there were no differences in solubility between brown-rotted and white-rotted bark (Table V). No attempts were made to determine the components of any of the extractives, and therefore it was not possible to identify the substrates that the fungi were using.

Fomes annosus and *S. lignicola* caused nearly twice as much weight loss from the nonextracted bark in the final experiment (Table IV) as they had in the previous experiment (Table III). The only difference in the two experiments was the autoclaving of the bark separately from the chambers in the final experiment. The bark in this case would have been subjected to higher temperatures for a longer time than when the bark was on the soil surface. Therefore, these two fungi appear to use a substrate that is made available by higher temperatures than was the substrate used by *L. saepiaria.*

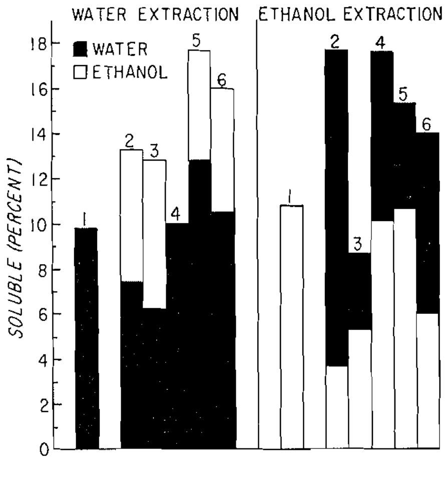

FIG. 2. Percentage water or ethanol extractives present in freshly ground stem bark of loblolly pine (1). Portions of each residue from (1) were air-dried (2) and oven-dried. The oven-dried portion was further divided into the following treatments: not autoclaved (3), autoclaved 45 min (4), autoclaved for two 45-min periods (5), and gas-sterilized with ethylene oxide (6). Each of the subtreatments was then subjected to nonexhaustive extraction, first with the original solvent and then with the other solvent.

TABLE V

Solubility (%) of stem bark of loblolly pine in 1% sodium hydroxide after various treatments and incubation for 12 weeks with *Fomes annosus*, *Lenzites saepiaria*, *Scytalidium lignicola*, or a noninoculated check

	Autoclave-sterilized			Gas-sterilized
Fungus	Water-extracted	Ethanol-extracted	Non-extracted	Non-extracted
Fomes annosus	72	66	67	67
Lenzites saepiaria	74	65	69	—
Scytalidium lignicola	71	64	61	67
Noninoculated check	51	62	63	62

TABLE VI

Properties of stem bark of loblolly pine

Property	Value, %	Source of information
Equilibrium moisture content	21–23 at 93% R.H.	Ref. 17
Saturation point	144 (Reduced pressure)	Author
Carbon content	48.4, 50.9	Author, ref. 1
Nitrogen content	0.137, 0.082, 0.421	Author, refs. 1, 18
Available carbohydrates	3–5	Author
Extractives		
(*a*) Ethyl alcohol soluble	9.6	Author
(*b*) Water soluble	9.5	Author
(*c*) 1% sodium hydroxide	63.0	Author

High C/N ratios may be responsible for some of the resistance of wood to decay (16). The bark used in this study had more N (C:N ratio = 353/1) than wood from the same trees (685/1, Table VI). Allison *et al.* (1) had found C/N ratios of 621/1 and 716/1 for bark and wood of loblolly pine, respectively. Metz and Wells (18) reported the average N content of loblolly pine bark in the Piedmont of South Carolina was more than three times larger than either of these two reports. Although the small amounts of N in bark may contribute to its low decay rate, N cannot be considered limiting unless it was demonstrated that N present is not available to the fungi.

This study has demonstrated that the bark is a poor substrate for sustained growth of many fungi, including wood rotters. Although most of the fungi tested grew luxuriantly on the surface of the bark cylinders, they were unable to cause biologically important increases in weight loss after incubation for 6 weeks. Under natural conditions, simple compounds are probably used rapidly as they become available, so that there would be little available for long term colonization by *F. annosus*. Previously, the importance of bark on pine roots as a barrier to infection of stump roots had been assumed from the observation that axe wounds through the bark caused a 10× increase in infection by spores of *F. annosus* (14). Similarly, mycelium of *F. annosus* must rely on woody substrates for energy to develop epiphytically on the root bark. In terms of survival of *F. annosus*, bark appears to serve primarily as a protective covering against drastic environmental changes in the woody tissues. After death of the root, all soil-borne organisms have ready access to the wood; however, the primary wood invaders are able to maintain dominance under the relatively stable environmental conditions assured by the bark.

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